

TITLE: METHOD AND APPARATUS FOR
AUTOMATICALLY ...
INVENTORS: Kensaku Imai et al.
SERIAL NO.: 09/785,269
DOCKET NO.: 826.1335C
Replacement Sheet filed 12/31/03

O I P E JCT 1
DEC 31 2003
PATENT & TRADEMARK OFFICE

SEQ ID: 5

M13mp18 (7250 bp)

6231

GAATTCGAGCTCGGTACCCGGGGATCCTCTAAGTCGACCTG CAG GCAT GCAAGCTT

EcoRI SacI KpnI SmaI BamHI XbaI SalI PstI SphI HindIII

SEQ ID: 6

M13mp19 (7250 bp)

AAGCTTG CATGCCTGCAGGTCGACTCTAGAGGATCCCCGGTACCGAGCTCGAATT

HindIII SphI PstI SalI XbaI BamHI SmaI KpnI SacI EcoRI

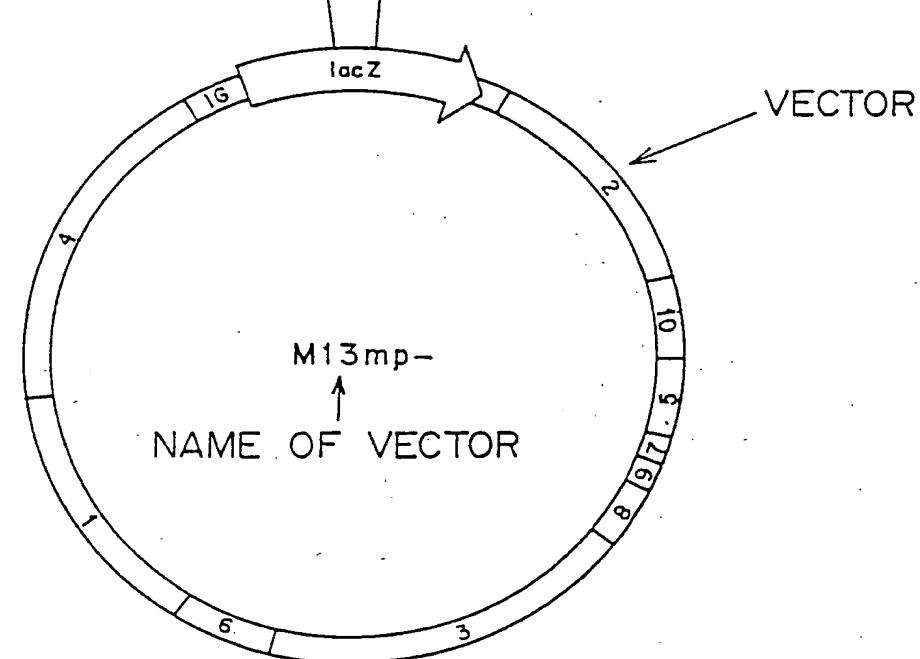


FIG. 3

DNA FRAGMENT LABELED WITH ^{32}P AT 5' EDGE

SEQ ID: 7 $^{32}\text{P}-\text{TGGCACTTGAAACGGCATGCT}$

RADIOACTIVE FRAGMENTS OF VARIOUS LENGTHS THROUGH CHEMICAL PROCESS OF SPECIFIC CLEAVING WITH RESIDUAL BASE A

SEQ ID: 8 $^{32}\text{P}-\text{TGCACCTGAAACGC}$ TGCT

SEQ ID: 9 $^{32}\text{P}-\text{TGCACCTGTA}$ CGCATGCT

SEQ ID: 9 $^{32}\text{P}-\text{TGCACCTTG}$ ACGCATGCT

SEQ ID: 10 $^{32}\text{P}-\text{TGCACCTTG}$ CTTGAACGGCATGCT

RADIOACTIVE UNLabeled FRAGMENT

THESE FRAGMENTS CAN BE STRICTLY ISOLATED DEPENDING ON LENGTH THROUGH GEL ELECTROPHORESIS

GEL

ELECTROPHORESIS

O I P E 30111
DEC 31 2003
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FIG. 4



VECTOR DB FORMAT

>ID
PUC18
>SEQ ID: 11
TCGC CGCTT CGGT GAT GAC CGGT GAAA ACCT CTGAC ACAT GCA GCT CCC GGAG AC CGGT ACAG CT GT CT GT AAG CGG AT
GCC GGG AGC AGA CAG ACC CGT CAG GCG CGT CAG CGG GT GT GG CGG GT GT CGG GT TA ACT AT CGG C AT CAGA
GCAG AT TG ACT GAG AGT GCA CC AT AT CGG GT GT GAA AT ACC CG C ACAG AT GCG TA AAG GAG AAA AT ACC CG C AT CAG GCG
AT TC GGC AT TC AGG GT GCG CA ACT GT TGG GAA AGG CG AT CGG GT CGG G C CT TT CG CT ATT AC G CAG CT GG GAA AGG
GG AT GT GCT GCA AGG CG AT TA AG GT TGG G TA AC G C C AGG GT TT CC CAG T CAC G AC GT GT AAA AC G AC G G C AGT G C C A A
GCT TGC AT G C C T G CAG GT CG ACT CT AG AGG AT C C C CG GT TAC CG GAG CT CG A AT TC G TA AT C AT GGT C AT AG CT GT T T C T
GT GT GAA AT TG IT AT CG CT CAC A AT CC AC AC AT AC GAG CG GAG C AT AA AGT GT TAA AG C CT GGG GT G C C T A AT G
AGT GAG CT A ACT CAC AT AAT CG GT T GCG CT ACT G C C CG CT TT CG AGT CG G G AA AC CT GT CG T G C C AG CT G C AT TA AT
GA AT CG G C C A AC G C G G G G AG AG G CG G TT T CG GT TAT GGG CG CT TT CG CT C C T CG CT C ACT G ACT CG CT G C G CT CG
GTC GT T CG G CT G C G G AG CG GT TAT CAG CT CACT CAA AGG CG GT TAT CGG TT AT CC AC AGA AT CAG G G AT A AC G C AG
AA AG A AC AT GT GAG CAA AGG C CAG C AAA AGG C CAG G AA ACC GT AAA AGG CG CG T T G C T G G C T T T C C AT AGG CT C C
G C C C C C T G C G AG C AG C AT CAC AAA AA AT CG AC G CT CA AGT CAG AGG T G G C G AA ACC CG AC AGG AT TA AG A AT ACC AGG CG
TT T C C C C T G G A AG C T C C C T G C G C T T T C T C A A A G C T C A C G C T G T A G G T A T C T C A G T C G G C T G T C A G T C G
G G G A A G C G T G G C G T T T C T C A A A G C T C A C G C T G T A G G T A T C T C A G T C G G C T G T C A G T C G C T G T C
T G C A C G A A C C C C C G T T C A G C C C G A C C G C T G C G C C T T A T C C G G T A A C T A T C G T C T T G A G T C C A A C C G G T A A G A C A C G A C
T T A T C G C C A C T G G C A G C A G C C A C T G G T A A C A G G A T T A C G A G G C G A G G T A T G T A G G C C G T G C T A C A G A G T T C T T G A A G T G
G T G G C C T A A C T A C G G C T A C A T G A A G A A C A G T A T T T G G T A T C T G C G C T G C G C T G C G C T G C G C T G
T T G G T A G C T C T T G A T C C C G G A A A C C A A C C C C G C T G G T A G G C C G T G G T T T T T G C A A G C C A G C A G A T A C C G C G A G A
A A A A A G G A T C T C A A G A A G T C T T G A T C T T C A C G G G T C T G A C G C T C A G T G G A A C G A A A A C T C A C G T T A A G G G A T
T T T G G T A C T G A G A T T A C A A A A G G A T C T T C A C C T A G A T C T T T A A A T T A A A A A T G A A G T T T T A A A T C A A T C T A A A G T A
T A T A T G A G T A A A C T T G G T C T G A C A G T T A C C A A T G C T T A A T C A G T G A G G C A C C T A T C T C A G C G A T C T G C T C A T T T C G T T C A
T C C A T A G T T G C C T G A C T C C C C G T C G T G T A G A T A A C T A C G A T A C G G G A G G G C T T A C C A T C T G G C C C C A G T G C T G C A A T G A T
A C C G C G A G A C C C A C G C T C A C C G G C T C C A G A T T T A T C A G C A A T A A A C C A G C C G G A A G G G C C G A G C G C A G A A G T G G T C
C T G C A A C T T T A T C C G C C T C A C C A G T C T A T T A A T T G T G C C G G A A G G C T A G A G T A A G T G A T T C G C C A G T T A A T A G T T G
C G C A A C G T T G T T G C C A T T G C T A C A G G C A T C G T G G G T C A C G C T C G T C G T T G G T A T G G C T T C A T T C A G C T C C G G T T C C C A
A C G A T C A A G G C G A G T T A C A T G A T C C C C A T G T T G C A A A A A G C G G T T A G C T C C T T C G G T C C T C C G A T C G T T G T C A G A A
G T A A G T T G G C C G A C T G T T A T C A C T C A T G G T T A T G G C A G C A C T G C A T A A T T C T C T T A C T G T C A T G C C A T C C G T A A A G T G C
T T T C T G T G A C T G G T G A G T A C T C A A C C A A G T C A T T C T G A G A A T A G T G T A T G C G G C G A C C G A G T T G C T C T T G C C C C G G C G T C
A A T A C G G G A T A A C C G C C C A C A T A G C A G A A C T T T A A A A G T G C T C A T C T T G G A A A A C G T T C T T C G G G G C G A A A A C T C T
C A A G G A T C T T A C C G C T G T G A G A T C C A G T T C G A T G T A A C C C A C T C G T G C A C C C A A C T G A T C T T C A G C A T C T T T A C T T T C
A C C A G C G T T T C T G G G T G A G C A A A A C A G G A A G G C A A A A T G C C G C A A A A A G G G A A T A A G G G C G A C A C G G A A A T G T G A A T
A C T C A A C T C T C C T T T T C A A T A T T A G A A G C A T T T A T C A G G G T T A T T G T C T C A T G A G C G G A T A C A T A T T G A A T G T A
T T T A G A A A A A A A C A A A T A G G G G T T C C G C G C A C A T T T C C C G A A A A G T G C C A C C T G A C G T C T A A G G A A C C A T T A T C
A T G A C A T T A A C C T A T A A A A T A G G C G T A T C A C G A G G C C C T T C G T C

>MULTI
399..450

FIG. 9

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(* INDICATES MULTIPLE CLONING SITE)

SEQ ID: 12 GTGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCTGTAAT

SEQ ID: 13 AACCTT \Rightarrow HIND III

SEQ ID: 14 GCATGC \Rightarrow SPH I

SEQ ID: 15 CTGCAG \Rightarrow PST I

SEQ ID: 16 GTCCGAC \Rightarrow SAL I,ACC I,HINC II

SEQ ID: 17 TCTAGA \Rightarrow XBA I

SEQ ID: 18 GGATCC \Rightarrow BAMH I

SEQ ID: 19 CCCGGG \Rightarrow SMA I,XMA I

SEQ ID: 20 GGTACC \Rightarrow KPN I

SEQ ID: 21 GAGCTC \Rightarrow SAC I

SEQ ID: 22 GAATTC \Rightarrow ECOR I

FIG. 10





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WHEN HIND III IS SPECIFIED ON VECTOR 5' SIDE
XBA I IS SPECIFIED ON VECTOR 3' SIDE, HIND III IS
SPECIFIED ON OBJECT DNA 5' SIDE, AND XBA I IS
SPECIFIED ON OBJECT DNA 3' SIDE

(***** INDICATES RESIDUAL MULTIPLECLONING SITE
(++++ INDICATES AN OBJECT DNA FRAGMENT

(SEQ ID NO. 4)

(SEQ ID NO. 23)

5' SIDE RETRIEVAL KEY
(IN THIS EXAMPLE,
HIND III SITE)

9' SIDE RETRIEVAL KEY
(IN THIS EXAMPLE, XBA I SITE)

FIG. 17